

GENETIC CONTROL OF FRUIT RIPENING

This invention relates generally to the modification of a plant phenotype by the regulation of plant gene expression. More specifically it relates to the modulation of the ripening and/or tissue senescence characteristics and plants derived therefrom. One suitable application of the present invention is the modulation of ripening and/or senescence processes in plants of the genus *Musa* (referred to herein as banana).

Two principal methods for the control of expression are known, viz.: overexpression and underexpression. Overexpression may be achieved by insertion of one or more than one extra copies of the selected gene. It is, however, not unknown for plants or their progeny, originally transformed with one or more than one extra copy of a nucleotide sequence, to exhibit the effects of underexpression as well as overexpression.

For underexpression, often referred to as "gene silencing", there are two principle methods which are commonly referred to in the art as "antisense downregulation" and "sense downregulation (also referred to as "cosuppression"). Both of these methods lead to an inhibition of expression of the target gene. Other lesser used methods involve modification of the genetic control elements, the promoter and control sequences, to achieve greater or lesser expression of an inserted gene.

There is no reason to doubt the operability of these methods: they are well- established, used routinely in laboratories around the world and products in which they have been used are on the market.

Gene control by any of these methods requires the insertion of a selected gene or genes into plant material which can be regenerated into plants. This transformation process can be performed via a number of methods, for example: the *Agrobacterium*-mediated transformation method.

In the microparticle bombardment method, microparticles of dense material, usually gold or tungsten, are fired at high velocity at the target cells where they penetrate the cells, opening an aperture in the cell wall through which DNA may enter. The DNA may be coated on to the microparticles or may be added to the culture medium.

In microinjection, the DNA is inserted by injection into individual cells via an ultrafine hollow needle.

Another method, viz. fibre-mediated transformation, applicable to both monocots and dicots, involves creating a suspension of the target cells in a liquid, adding microscopic needle-like material, such as silicon carbide or silicon nitride "whiskers", and agitating so that the cells and whiskers collide and DNA present in the liquid enters the cell.

5 In summary, then, the requirements for both sense and antisense technology are known and the methods by which the required sequences may be introduced are known. What remains, then is to identify genes whose regulation will be expected to have a desired effect, isolate them or isolate a fragment of sufficiently effective length, construct a chimeric gene in which the effective fragment is inserted between promoter and termination signals, and insert the construct into cells of the target plant species by transformation. Whole plants may then be regenerated from the transformed cells.

10 Bananas are a globally important fruit crop. They are not only a popular dessert fruit, but represent a vital carbohydrate staple in the tropics with as many as 100 million people subsisting on bananas and plantains as their main energy source. The cultivated dessert banana is commonly triploid, parthenocarpic and belongs to the *Musa* AAA genome group, eg. Cavendish subtypes. Bananas are climacteric fruits and ripening is regulated by ethylene produced by the fruit and involves numerous biochemical changes including the conversion of starch to sugars, cell wall disassembly, synthesis of volatile compounds, changes in
15 phenolic constituents and degradation of chlorophyll in the peel. The conversion of starch to sugars is particularly striking, where starch accounts for 20-25% of the fresh weight of the unripe fruit and depending on the genetic background, can be converted almost entirely to sugars.

20 The triploid nature of the cultivated dessert banana crop has hampered conventional methods of breeding for improved characteristics. As a result of this an enormous pool of genetic resources for enhancing postharvest characteristics of the fruit has remained untapped.

According to the present invention there is provided a method of modulating the ripening or tissue senescence process in plants of the genus *Musa* comprising inserting into plant
30 material at least one polynucleotide sequence selected from the sequences depicted as SEQ ID-Nos. 1-57 ^{SEQ ID NOS: 1-57} regenerating said plant material and selecting from the transformed

regenerants, plants with modulated ripening or tissue senescence characteristics. The said polynucleotide may be obtained from the cDNA library having the NCIMB Accession Number 40814.

Further according to the present invention is a method of modulating the ripening or tissue senescence process in plants of the genus *Musa* comprising inserting into plant material at least one polynucleotide sequence or a fragment thereof, obtainable by hybridisation, from the cDNA library having the NCIMB Accession Number 40814, by the use of at least one of the sequences depicted as [SEQ ID Nos 1-57] ^{✓ SEQ ID NOS: 1-57} as oligonucleotide probes, said hybridisation being conducted at a temperature from 60°C to 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS, regenerating said plant material and selecting from the transformed regenerants, plants with modulated ripening or tissue senescence characteristics. The invention further provides a method as described above wherein the said polynucleotide is capable of modulating the production of pectate lyase and more specifically the polynucleotide comprises at least one of the sequences depicted in the sequence listings as [SEQ-ID-Nos. 13-18] ^{✓ SEQ ID NOS: 13-18}

A preferred method for inserting the said polynucleotides into plant material according to the method of the present invention, may be selected from the group comprising the *Agrobacterium*, microparticle bombardment, fibre mediated or direct insertion methods.

The invention further provides plants, their progeny and seed and material obtained from said plants, produced according to the method of the present invention. It is preferred that the said plants, their progeny and seed and material obtained from said plants are derived from plants of the genus *Musa*.

The present invention also provides a vector functional in plants comprising a promoter region which is operable in plant cells, at least one of the polynucleotide sequences described above and a transcription termination sequence. The promoter may be constitutive, developmentally regulated or switchable. In addition to this the promoter may also be tissue specific or organ specific.

Further provided is a banana produced via the preceding method, having altered fruit characteristics when compared with a banana which is not transformed with at least one of the polynucleotide sequences described above.